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A. Cobelo-García, A. Tovar-Sánchez,
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Seasonal evolution of cholorophyll-*a* and cyanobacteria (*Prochlorococcus* and *Synechococcus)* on the northeast continental shelf of the Gulf of Cádiz: relation to thermohaline and nutrients fields

ELEONORA ANFUSO [1], BIBIANA DEBELIUS [1], CARMEN G. CASTRO [2], ROCIO PONCE [1], JESUS M. FORJA [1] and LUIS M. LUBIAN [3]

[1] Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Campus Río San Pedro s/n, 11510 Puerto Real, Cádiz, Spain. E-mail: anfusoeleonora@gmail.com
[2] Departamento de Oceanografía, Instituto de Investigaciones Marinas, CSIC, c/ Eduardo Cabello 6, 36208 Vigo, Pontevedra, Spain.
[3] Instituto de Ciencias Marinas de Andalucia, CSIC, 11510 Puerto Real, Cádiz, Spain.

SUMMARY: The seasonal evolution of nutrients, chlorophyll-*a* (chl-*a*) and cyanobacteria (*Synechococcus* and *Prochlorococcus*) on the northeast continental shelf of the Gulf of Cádiz was observed during four cruises in summer and autumn 2006 and winter and spring 2007. Samples were collected to determine the distribution of chl-*a* and cyanobacteria abundance and to analyse their coupling with thermohaline and chemical properties in the gulf region. Surface nutrient distributions showed clear seasonal variability. Maximum levels were recorded for the entire study region during winter due to winter mixing and the strong influence of the Guadalquivir River and Bay of Cádiz outflow. Maximum chl-*a* levels were reached during spring for the entire region and for the entire water column. Minimum values of chl-*a* were observed during summer, under prevailing oligotrophic conditions, and winter when in spite of relatively high nutrient concentrations, phytoplankton growth was probably light-limited. The distribution of both cyanobacteria populations also varied seasonally in association with the oceanographic conditions. *Prochlorococcus* maximum cell abundance was observed at the sea surface during the four cruises, except in the northern region during summer. However, *Synechococcus* showed a maximum concentration at the sea surface in autumn and winter and at the subsurface chlorophyll maximum in autumn and spring.

Keywords: chlorophyll-*a*, cyanobacteria, seasonal evolution, Guadalquivir River, Gulf of Cádiz.

RESUMEN: Evolución estacional de clorofila-a, y cianobacteria (*Prochlorococcus* y *Synechococcus*) en la parte noroccidental de la plataforma continental del golfo de Cádiz: relación con las propiedades termohalinas y de nutrientes. – Durante cuatro campañas, verano y otoño 2006 e invierno y primavera 2007, se examinó la evolución estacional de nutrientes, clorofila-*a* (chl-*a*) y cianobacterias en la parte noroccidental de la plataforma continental del Golfo de Cádiz. Se tomaron muestras para el analisis de la distribución de chl-*a* y cianobacterias y para analizar su acoplamiento con las propiedades termohalinas y químicas en la región del golfo. La distribución superficial de nutrientes presentaba una clara estacionalidad, de hecho las máximas concentraciones se registraron en toda el área de estudio durante el invierno debido a la mezcla invernal y a la fuerte influencia del los aportes del rio Guadalquivir y la Bahía de Cádiz. Los máximos niveles de chl-*a* se alcanzaron en primavera en toda la región y en toda la columna de agua. Mínimos valores de chl-*a* se observaron en verano, cuando prevalecían condiciones oligotróficas y en invierno cuando, a pesar de una alta concentración de nutrientes, el crecimiento del fitoplancton estaba limitado por la luz. La distribución de ambas poblaciones de cianobacterias varió tambien estacionalmente asociada a las condiciones oceanográficas. La máxima abundancia de células de *Prochlorococcus* se observó en aguas superficiales durante las cuatro campañas, a excepción de la región norte en verano. Por otro lado, *Synechococcus* presentó la maxima concentración en aguas superficiales en otoño y invierno y en el máximo de clorofila subsuperficial en otoño y primavera.

Palabras claves: clorofila-*a*, cyanobacteria, evolución estacional, rio Guadalquivir, Golfo de Cádiz.

INTRODUCTION

Ocean phytoplankton is responsible for approximately half of the global biosphere net primary production (Behrenfield *et al.* 2001). About 25% of total oceanic primary production occurs on the continental shelves (Wollast 2002), even though they only represent 7% of the oceanic surface. The high primary production of the continental shelves is related to a relatively high nutrient input from continental sources, upwelled nutrient-rich subsurface waters and exchange with sediments. Changes in chlorophyll-*a* and primary production associated with thermohaline and biogeochemical properties (inorganic and organic nutrients) have been well studied in some coastal, mainly upwelling, regions (Raimbault *et al.* 1988, Ruiz *et al.* 1996 and Sabetta *et al.* 2008). However, there are other coastal systems that have not received very much attention until recently, such as the Gulf of Cádiz.

The Gulf of Cádiz is a basin between the Iberian Peninsula and the African Continent, in the SW part of Spain (Fig. 1). The circulation in the region is regulated by winds and also by the varying presence of waters from the Mediterranean Sea and Atlantic Ocean (Navarro and Ruiz 2006). During summer, the circulation in the region is mainly anticyclonic (García *et al.* 2002, Criado-Aldeanueva *et al.* 2006a, b), shifting to cyclonic during winter (García-Lafuente and Ruiz 2007). Some characteristic mesoscale structures have been identified during the summer period: the "Huelva Front", a warm-cold-warm area southeastward of Cape Santa María (Stevenson 1977), the "Portuguese upwelling zone" close to Cape San Vicente, and the "Tarifa eddy", a mixing zone at Cape Trafalgar (Fiuza *et al.* 1982, Fiuza 1983, Folkard *et al.* 1997). Different water masses have been described in the Gulf of Cádiz: Surface Atlantic Water (SAW) and Surface Water (SW), located in the central part of the basin and over the continental shelf respectively; North Atlantic Central Water (NACW), between 100 and 700 m depth (Folkard *et al*. 1997); and Mediterranean Water (MW) below 700 m depth.

Previous studies on the Gulf of Cádiz have shown the seasonal horizontal distribution of chl-*a* at the sea surface based on in situ measurements (Prieto *et al*. 2009) or based on remote sensing images (Navarro *et al*. 2007 and Navarro and Ruiz 2006). Prieto *et al.* (2009) described high chl-*a* levels in the vicinity of the Guadalquivir river mouth during the spring months. Navarro *et al.* (2007) reported a phytoplankton bloom from November to April, in agreement with Navarro and Ruiz (2006), who observed a chl-*a* maximum in spring and autumn in the coastal zone. However, the vertical distribution of chl-*a* in the gulf region has not been so well studied. There are only a few studies on the vertical distributions of chl-*a* during spring (Navarro *et al*. 2006) and summer (García *et al*. 2002; Prieto *et al*. 1999). In addition, Echevarría *et al.* (2009) found high levels of *Prochlorococcus* and *Synechococcus* in the Gulf of Cádiz associated with the subsurface chl-*a* maximum for a summer cruise. Except for these papers, there are no previous studies on the vertical distribution of chl-*a* and its coupling with the thermohaline and chemical properties for the gulf region. The aim of our study is to examine, for the first time, the spatial variation of chl-*a* and cyanobacteria in relation to thermohaline and nutrient distributions in the NE continental shelf of the Gulf of Cádiz on a seasonal basis.

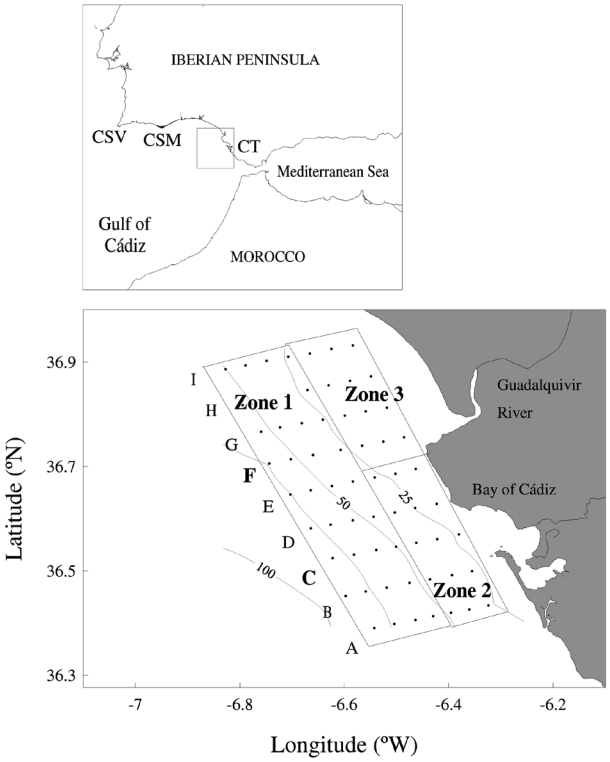

FIG. 1. – Map of the northeastern continental shelf of the Gulf of Cádiz (SW Iberian Peninsula). Solid points indicate sampling stations for the EMIGAS I (summer), II (autumn), III (winter) and IV (spring) cruises. Letters from 'A' to 'I' correspond to transect labels. The three zones into which the study area is divided are also shown: zone 1, corresponding to the oceanic area, zone 2, corresponding to the southern area, and zone 3, corresponding to the northern area. CSV, CSM and CT stand for Cape San Vicente, Cape Santa Maria and Cape Trafalgar respectively. Isobaths are in metres.

MATERIALS AND METHODS

The study area was located on the northeastern shelf of the Gulf of Cádiz (SW Iberian Peninsula) (Fig. 1). The sampling stations were selected covering an area from the Bay of Cádiz to the Guadalquivir River. The data were collected during four oceanographic cruises (EMIGAS I, II, III and IV) carried out in summer (June 2006), autumn (November 2006), and winter (January 2007) on board R/V *Mytilus,* and in spring (May 2007) on board R/V *UCádiz*. A grid of 63 stations, divided into nine transects, was sampled. At each station, continuous vertical profiles of temperature, salinity, pressure and fluorescence were obtained with a

Seabird CTD probe coupled to a Seatech fluorometer and a Seabird 43 dissolved oxygen sensor. Discrete water samples were collected for analysis of nutrients, chl-*a* and cyanobacteria at different depths in the water column. For nutrients and chl-*a*, water samples were collected at the sea surface, 20 m and at 2 m above the bottom depending on station bathymetry. For cyanobacteria, samples were collected at the sea surface and at the subsurface chlorophyll maximum (SCM) depth. This depth was established based on the down CTD cast and it varied from a minimum of 6 m at the inshore stations to a maximum depth of 87 m at an offshore station. Whenever the chlorophyll maximum was located at the sea surface, we collected only one cyanobacteria sample at the sea surface.

Nutrient samples were filtered on board through 0.45 μm GF/F Millipore filters, immediately frozen at –20°C and analysed in the laboratory. Nutrient concentrations were determined by segment flow analysis with Alpkem autoanalysers following Grassolff *et al.* (1983). The analytical errors were ±0.05 μM for nitrate and silicate, and ±0.01 μM for phosphate. For chl-*a* analysis, about 500 mL was filtered on board using 0.45 μm GF/FMillipore filters, frozen at –20°C and then analysed in the laboratory by fluorometry after extraction with 90% acetone in the dark over night. For the cyanobacteria, about 100 mL was collected in an amber bottle and fixed with 30% formaldehyde, and immediately analysed once back in the laboratory. The samples were analysed using a FACSCalibur (Becton-Dickinson) flow cytometer, equipped with a blue argon-laser (488 nm), a three colour photomultiplier with fluorescence emission filters (FL1 515-545 nm, FL2 564-606 nm, FL3>650 nm) forward light scatter (FSC) and side scatter (SSC), a red diode laser (635 nm) and a fourth coloured photomultiplier with fluorescence emission filters (FL4 653-669 nm). A known volume of each sample was analysed and data were computed with CellQuest software (Beckton-Dickinson). The SSC signal was used as the cellular size indicator (Sobrino *et al*. 2004), and FL3, FL2 and FL4 were used as indicators of chlorophyll (Trask *et al.* 1982; Olson *et al.* 1985 and Yentsch *et al.* 1986), phycoerytrin and phycocyanin contents respectively. Each sample was analysed for 30-60 s (6000 to 10000 events per measurement, at a flow rate previously calibrated with an established sample weight during a constant period of time). Cell abundance was transformed to carbon content (pg-C mL^{-1}) according to DuRand *et al.* (2001) using the ratios 0.112 and 0.056 pg-C $cell^{-1}$ for *Synechococcus* and *Procholorochoccus* respectively.

Stability of the water column was evaluated by means of the integrated Brunt-Vaisala frequency (N^2):

$$N^2=g/z \ln (\rho_z/\rho_0)$$

where g is the gravitational acceleration, z is the water depth and ρ_z and ρ_0 the bottom and surface densities respectively.

Nitracline depth was defined as the shallowest depth at which the nitrate concentration was higher than 1 μM (Moran *et al*. 2001).

The data on the Guadalquivir River discharge were provided by courtesy of the Hydrographic Confederation of Guadalquivir.

Seasonal and zonal differences were analysed using a *t*-test statistical treatment for independent variables.

RESULTS

Horizontal superficial distribution

The thermohaline, chemical and biological fields varied widely for the four seasonal cruises (Figs 2, 3 and 4). Temperature showed strong variations during the four seasons. The highest temperature values (18.5-20°C and 21-24°C respectively) were reached in the spring and summer cruises, while minimum values were observed in winter (<13ºC). During spring and summer, the warmest waters were found close to the Guadalquivir River, establishing a clear oceanwards decreasing thermal gradient in the north region (zone 3 in Fig. 1), while surface temperatures were almost homogeneous in the south (zone 2 in Fig. 1). During winter, we observed a different situation, with a marked thermal front for the study area and minimum

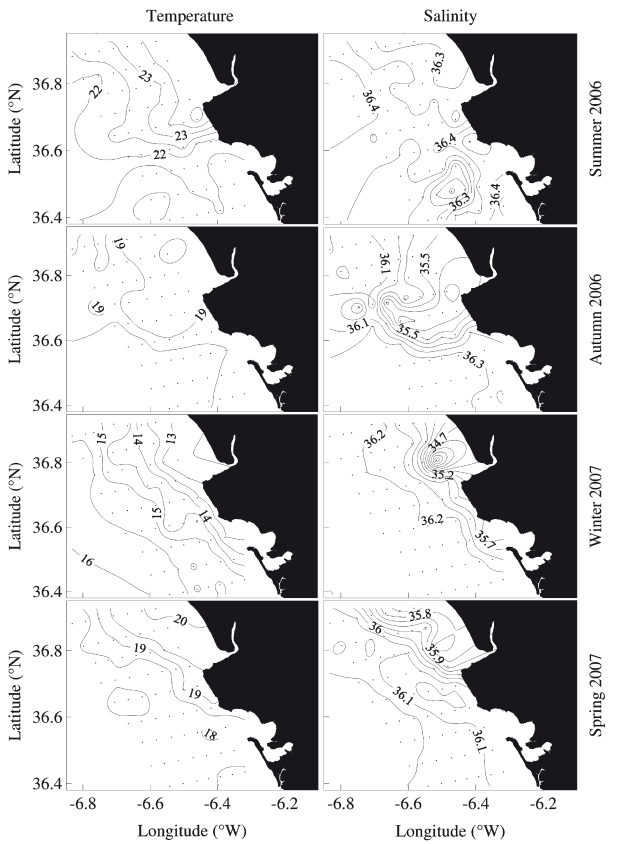

FIG. 2. – Horizontal distributions of sea surface temperature (°C) and salinity in the Gulf of Cádiz during the summer, autumn, winter and spring cruises.

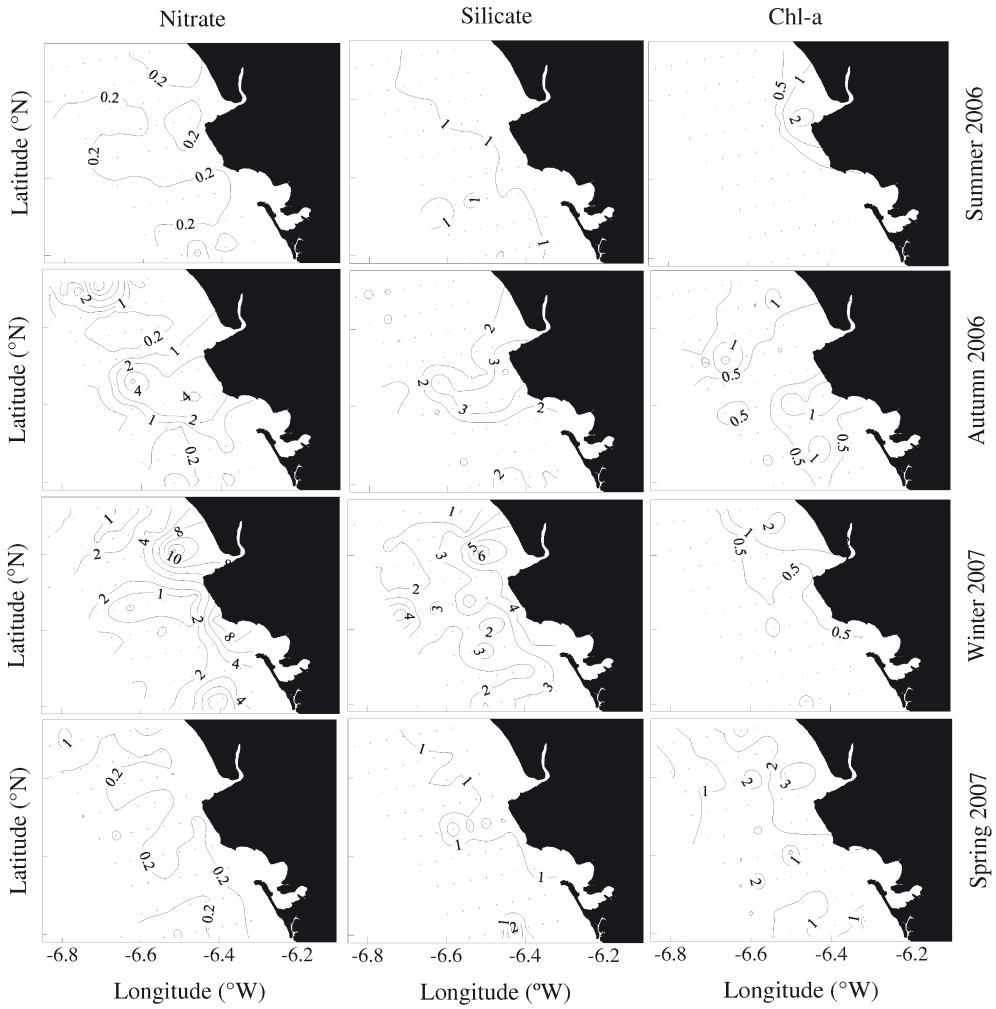

FIG. 3. – Horizontal distributions of chl-*a* (μg dm^{-3}) and nitrate (μM) in the Gulf of Cádiz during the summer, autumn, winter and spring cruises.

temperatures associated with the Guadalquivir plume. Finally, the autumn distribution was characterized by similar temperatures for the entire region (19.2±0.4°C) and the absence of a thermal front.

Salinity showed a clear oceanwards increasing gradient for all seasons except summer (Fig. 2), when there was no clear difference between the coastal and oceanic stations. For the three other seasons, the salinity field was controlled by the Guadalquivir River. During winter, the salinity front due to Guadalquivir outflow was closer to shore, while in spring and autumn, the influence of fresh waters (S<35.9) reached more southern and offshore stations due to the higher river discharge in these seasons (39.7 m^3 s^{-1} and 37.7 m^3 s^{-1} for autumn and spring respectively; data from Hydrographic Confederation of Guadalquivir).

Similarly, surface nutrient distributions followed a clear seasonal variability (Fig. 3 and Table 1). Maximum nutrient levels were observed for the entire study region during winter (nitrate and silicate levels>2 μM – Fig. 3; and phosphate >0.5 μM – distribution not shown) due to winter mixing and a strong continental outflow from the Guadalquivir River and Bay of Cádiz outflow. In fact, maximum nutrient levels for this period (14.7 μM for nitrate, 0.78 μM for phosphate and 7.4 μM for silicate) were associated with these continental outflows. This situation clearly contrasts with the summer and spring cruises, when nutrients reached minimum values (<0.5 μM, <1 μM and <0.2 μM for nitrate, silicate and phosphate respectively). Although autumn did not show nutrient values as high as those in winter, it was characterized by relatively high values with maximum nitrate and silicate values in the tongue of low salinity from the Guadalquivir River (NO_3=9.9 μM and SiO_2=4.2 μM; Fig. 3).

The distribution of chl-*a* (Fig. 3) showed high values during spring for the entire region (chl-*a*>1 μg dm^{-3}), in contrast to the other three seasons, with an average surface chl-*a* of 0.15±0.35 μg dm^{-3}, 0.51±0.48 μg dm^{-3} and 0.42±0.41 μg dm^{-3} for summer, autumn and winter respectively. For all seasons, except autumn, the chl-*a* maximum was located in the northern part with values as high as 2.60 μg dm^{-3}, 2.25 μg dm^{-3} and 3.60 μg dm^{-3} for summer, winter and spring respectively

TABLE 1. – Mean values and standard deviation of sea surface concentration of nutrients (μM) for the three zones shown in Figure 1 during the four samplings. Brackets include the maximum and minimum values (μM) for each sampling.

		Nitrate	Phosphate	Silicate
Summer 2006	Zone 1	0.27±0.38	0.09±0.04	0.88±0.14
		(2.44-0.00)	(0.18-0.01)	(1.22-0.57)
	Zone 2	0.23±0.14	0.06±0.03	0.97±0.21
		(0.58-0.00)	(0.10-0.00)	(1.27-0.48)
	Zone 3	0.21±0.04	0.15±0.04	1.34±0.23
		(0.30-0.16)	(0.22-0.09)	(1.91-1.03)
Autumn 2006	Zone 1	0.98±2.04	0.15±0.06	1.63±0.81
		(9.87-0.00)	(0.34-0.07)	(4.07-0.83)
	Zone 2	1.65±1.38	0.17±0.068	2.168±1.016
		(4.26-0.16)	(0.31-0.10)	(4.16-1.0)
	Zone 3	0.95±1.61	0.15±0.08	1.56±0.60
		(5.64-0.05)	(0.25-0.00)	(2.89-1.05)
Winter 2007	Zone 1	1.99±1.46	0.83±2.05	2.23±0.84
		(8.77-0.00)	(11.46-0.15)	(5.86-1.30)
	Zone 2	3.24±3.03	1.12±2.19	2.95±1.06
		(9.44-0.00)	(8.46-0.17)	(4.91-1.57)
	Zone 3	4.02±4.05	0.27±0.18	2.92±1.71
		(14.68-0.00)	(0.78-0.12)	(7.34-0.59)
Spring 2007	Zone 1	0.22±0.26	0.15±0.04	0.62±0.72
		(1.29-0.00)	(0.32-0.08)	(3.83-0.10)
	Zone 2	0.17±0.13	0.14±0.03	0.67±0.63
		(0.47-0.00)	(0.22-0.10)	(2.55-0.08)
	Zone 3	0.38±0.41	0.14±0.04	1.18±0.41
		(1.40-0.01)	(0.21-0.09)	(1.75-0.43)

Surface *Prochlorococcus* distributions (Fig. 4) showed high concentrations during summer (7.6±6.6 10^3 cell mL^{-1} or 482±366 pg-C mL^{-1}) and spring (43.0±15.7 10^3 cell mL^{-1} or 2407±881 pg-C mL^{-1}), decreasing in autumn and winter (3.6±2.2 10^3 cell mL^{-1} or 206±125 pg-C mL^{-1} and 6.1±3.7 10^3 cell mL^{-1} or 340±209 pg-C mL^{-1} respectively; Table 2). During summer, maximum values were observed at the inshore stations of the northern region (zone 3 in Fig. 1) with a water temperature >22°C. During spring, high values of *Prochlorococcus* cell abundance were observed for all stations, with maximum values (>70 10^3 cell mL^{-1}) in the northern transects, not only at the inshore stations (zone 3 in Fig. 1) but also at the most oceanic stations corresponding to zone 1. In autumn and winter, *Prochlorococcus* levels were much lower, with maximum levels (aprox. 10 10^3 cell mL^{-1}) at the offshore stations corresponding to the warmest waters for these periods. Conversely, sea surface distributions of *Synechococcus* (Fig. 4) showed the lowest values during summer (403±2710 cell mL^{-1} or 46±310 pg-C mL^{-1}) and spring (278±793 cell mL^{-1} or 31±88 pg-C mL^{-1}) and maximum values in winter (14.2±5.8 10^3 cell mL^{-1} or 1597±648 pg-C mL^{-1}) and autumn (3.8±3.6 10^3 cell mL^{-1} or 429±407 pg-C mL^{-1}). The highest concentrations (>10 10^3 cell mL^{-1}) were observed in the oceanic area (zone 1 in Fig. 1) in winter and autumn. During the summer and spring cruises, *Synechococcus* was practically absent at the sea surface except for at a northern oceanic station in summer, and a station close to the Bay of Cádiz in spring.

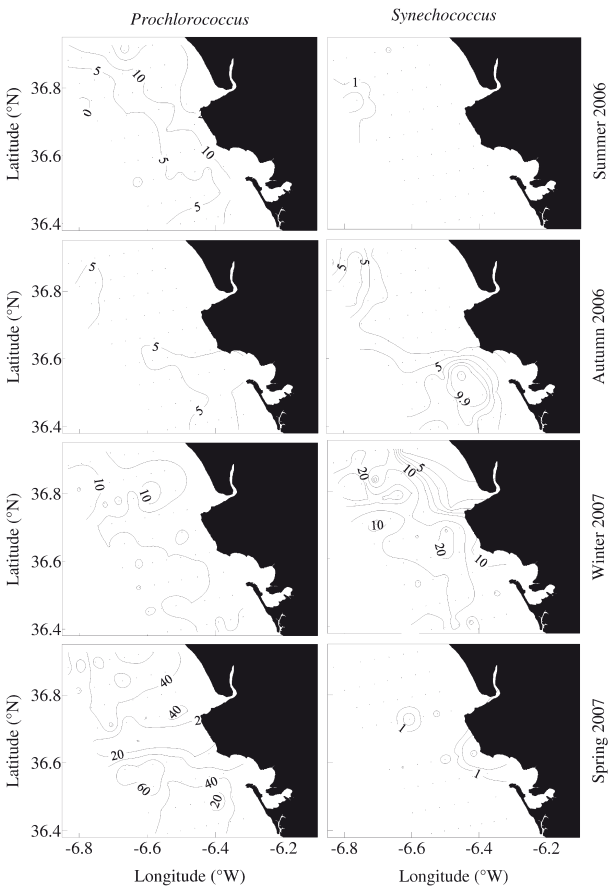

FIG. 4. – Horizontal distributions of *Prochlorococcus* (10^3 cell mL^{-1}) and *Synechococcus* (10^3 cell mL^{-1}) in the Gulf of Cádiz during the summer, autumn, winter and spring cruises.

TABLE 2. – Average values with standard deviation and maximum and minimum cell abundances for *Prochlorococcus* and *Synechococcus* at the sea surface (×10^3 cell mL^{-1}). The biomass is indicated in brackets (pg-C mL^{-1}).

	Prochlorococcus		*Synechococcus*	
	Average value	Maximum and minimum	Average value	Maximum and minimum
Summer	7.6±6.6	34.8-100	4.6±8.9	20.5-108
	(382±374)		(40±292)	
Autumn	3.6±2.2	9.3-701	3.7±3.6	17.6-172
	(203±125)		(422±407)	
Winter	6.0±3.7	26.6-0	14.2±5.7	26.4-451
	(340±207)		(1597±643)	
Spring	42.9±15.7	77.9-3740	278±793	4.5-0
	(2035±1212)		(33±81)	

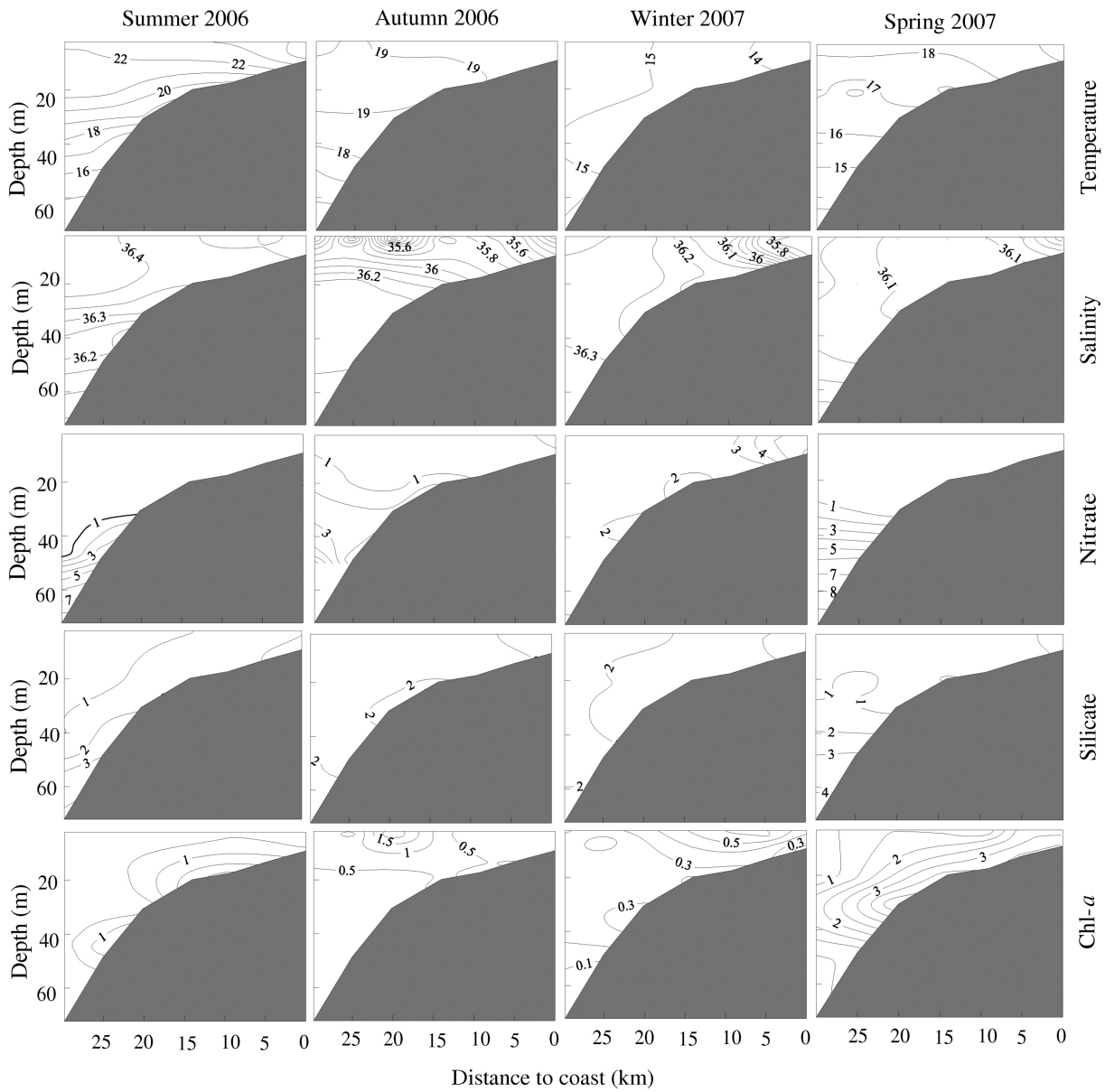

FIG. 5. – Vertical distributions of temperature (°C), salinity, chl-*a* (μg dm^{-3}), nitrate (μM) and silicate (μM) at transect F off Guadalquivir River during the summer, autumn, winter and spring cruises

Vertical distribution

We selected two different transects that best represent the study area, corresponding to the northern (transect F) and southern (transect C) regions related to the Guadalquivir River and the Bay of Cádiz respectively (Figs 5 and 6). In both transects the maximum temperature was recorded during summer, and then decreased during the other seasons, until it reached minimum values in winter. In summer we observed a temperature variation of approximately 4°C in the northern area, while it was 3.5°C in the southern area. During spring the stratification was not so strong and it was stronger in the southern than the northern region, with a temperature difference of 2°C and 2.5°C respectively. For autumn, the upper 40 meters of the water column was relatively warm (>19°C) and well mixed for the two transects. This part of the water column was still homogenized in zone 3 during winter though with colder temperatures (<14°C), while in zone 2 we observed an oceanwards increasing temperature trend.

The salinity distribution in zone 3 was clearly controlled by the influence of the Guadalquivir River for all seasons except summer. The river outflow reached the farthest offshore stations in autumn, as previously described for the horizontal distributions. In contrast the salinity distribution in zone 2 was characterized by a low gradient for the entire transect except for the low salinity values determined at the stations closest to the Bay of Cádiz during autumn and winter.

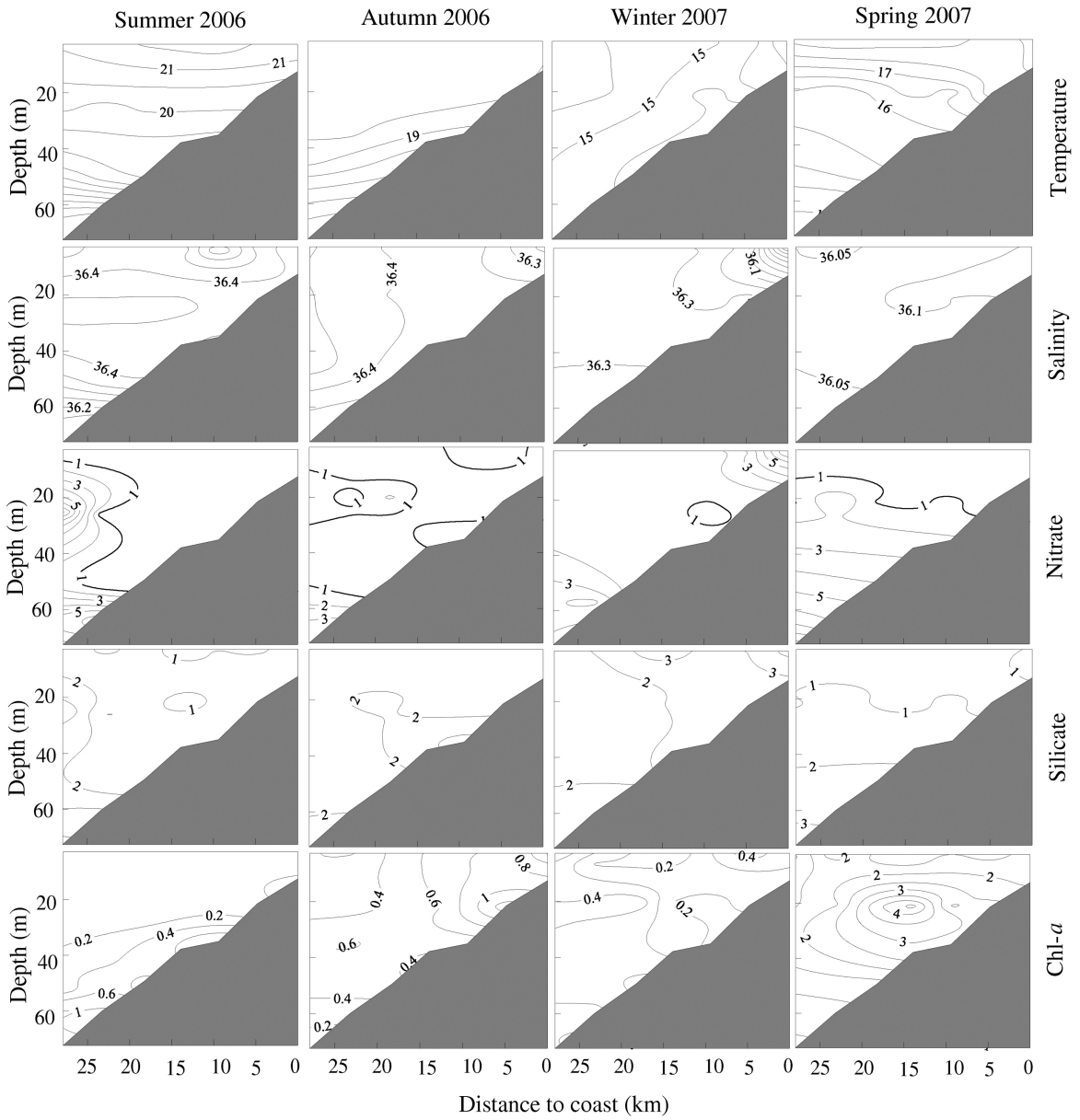

FIG. 6. – Vertical distributions of temperature (°C), salinity, chl-*a* (μg dm^{-3}), nitrate (μM) silicate (μM) at transect C off the Gulf of Cádiz during the summer, autumn, winter and spring cruises.

Nitrate vertical distributions (Fig. 5) respond to the thermohaline vertical distributions, as previously indicated for sea surface horizontal maps. During spring and summer, nitrate levels were completely depleted in the upper water column and the nitracline was located at an average depth of 32±11 m for summer and 25 meter for spring in zone 3. However, during winter we observed nitrate levels lower than 2 μM for all the stations, and the highest values were at the inner stations closest to the Guadalquivir River. An intermediate situation was observed in the autumn cruise, with a shallower nitracline depth than during spring and summer, and with higher surface nitrate levels (0.5-1 μM). In general, silicate (Fig. 5) and phosphate vertical distributions (not shown) were similar to the nitrate vertical distributions for all seasons, though the imprint of the Guadalquivir River was clearly traceable on the silicate vertical distributions. Vertical distributions of nutrients off the Bay of Cádiz (Fig. 6) showed a similar seasonal pattern to the northern distributions. Maximum nutrient concentrations for the entire water column were recorded in winter and surface depleted waters in summer and spring, as described for the northern section. However, relatively lower nutrient levels for this southern transect compared with the northern one were measured during autumn. The silicate contribution from the Bay of Cádiz continental waters was also clearly marked in the winter and spring cruises; however, we did not observe this signal in the autumn.

TABLE 3. – Percentage of stations with subsurface maximum cell abundance of *Prochlorococcus* and *Synechococcus* during each cruise. 'n' corresponds to the number of stations

	Prochlorococcus	*Synechococcus*	n
Summer	53%	98%	57
Autumn	21%	14%	63
Winter	11%	2%	62

The highest chl-*a* concentrations were recorded for the entire water column during spring for both transects (2.13±1.33 μg dm^{-3} for zone 3 and 2.30±1.07 μg dm^{-3} for zone 2). Chl-*a* levels decreased in summer reaching minimum values in autumn and winter (0.46±0.56 μg dm^{-3} and 0.50±0.32 μg dm^{-3}; 0.30±0.16 μg dm^{-3} and 0.24±0.11 μg dm^{-3} respectively for zone 3 and zone 2 for each season). During spring similar maximum values for both transects were recorded in the surface water and at nitracline depth. This vertical distribution of chl-*a* was still present during the summer cruise; however, chl-*a* levels were higher in the northern transect than in the southern transect. In autumn and winter, low chl-*a* values were observed in the entire water column in both regions.

As mentioned in the material and methods section, we only collected surface and subsurface chl-*a* maximum samples for *Prochlorococcus* and *Synechococcus* populations whenever there was an SCM. At those stations where the chl-*a* maximum was at the sea surface, we only sampled the sea surface bottle for cyanobacteria counts. Therefore, we compared the number of stations where the maximum cell abundance of *Prochlorococcus* and *Synechococcus* occurred at the SCM and not at the sea surface for each seasonal cruise (Table 3). Maximum cell abundance of *Prochlorococcus* was mainly at the sea surface in all seasons except summer. About 53% of the sampled stations showed higher values of *Prochlorococcus* at the SCM than at the sea surface during summer. *Synechococcus* showed a different seasonal pattern of vertical levels of cell abundances between the sea surface and the SCM. Maximum *Synechococcus* cell abundance was observed at the SCM during summer and spring, occurring in 98% and 87% of the sampled stations respectively (Table 3). In contrast, the maximum cell abundance was mainly located at the sea surface during the autumn and winter cruises.

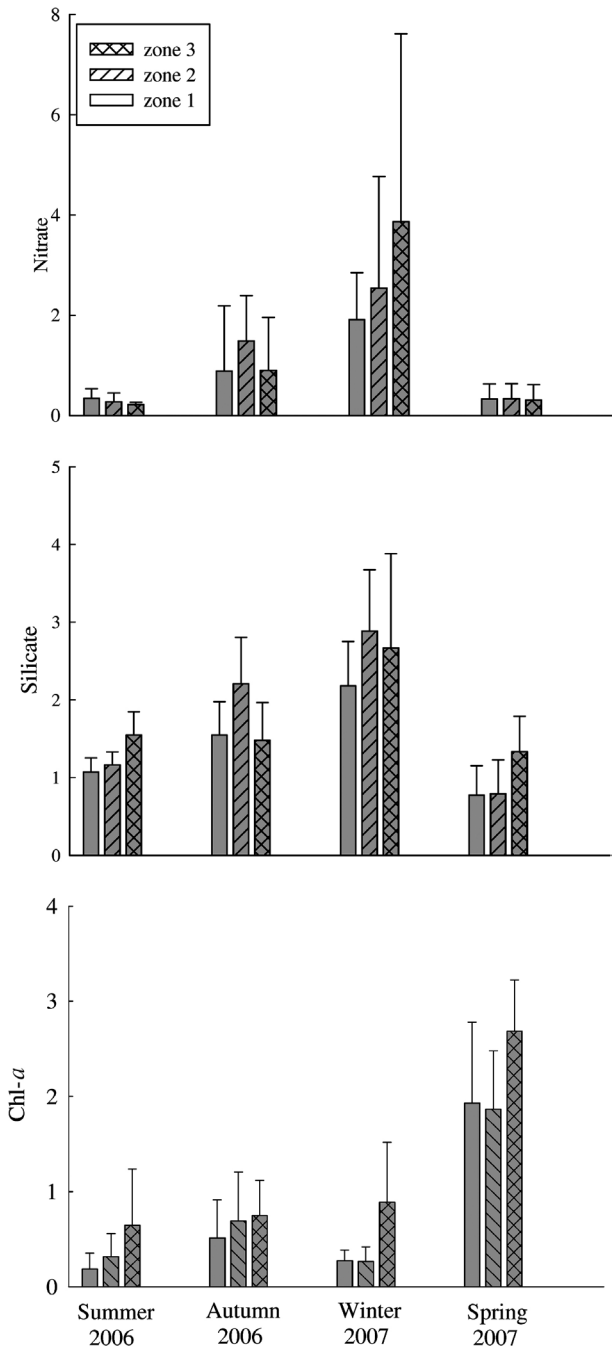

FIG. 7. – Seasonal average values of nitrate (μM), silicate (μM) and chl-*a* (μg dm^{-3}) between the sea surface and nitracline depth for the three zones. Zone 1 corresponds to the oceanic area while zones 2 and 3 indicate the south and north regions respectively (Fig. 1).

DISCUSSION

Spatial and seasonal distribution of chl-*a* and cyanobacteria

The Gulf of Cádiz is a basin that has different conditions during the year. As previously discussed by several authors (Garcia-Lafuente *et al.* 2006, Navarro and Ruiz 2006, Ruiz *et al.* 2006, Prieto *et al.* 2009), during our study year we observed the highest temperatures in summer and the coldest waters in winter, with significant differences between cruises (*t*-test, $p<0.01$) (Fig. 2), and extreme values recorded close to the Guadalquivir River. As observed in the salinity field for the four cruises, the Guadalquivir River plays a key role, clearly delimiting the coastal inshore stations south and north of the river (zones 2 and 3 respectively in Fig. 1). In fact, salinity averages for the three classified zones were significantly different for the four cruises except salinity averages for zone 1 and 2 in summer (*t*-test, $p<0.05$). Therefore, based on the

thermohaline properties, the study region can be subdivided into three zones: an 'oceanic' area (zone 1) and two coastal inshore areas (zone 2 and 3) divided by the Guadalquivir River.

The results presented in this work showed seasonal variation in nutrients, with maximum values in winter compared with the other seasons in each of the three regions ($p<0.01$). Minimum values were recorded during summer and spring, with average concentrations of nitrate, phosphate and silicate lower than 0.4 μM, 0.2 μM and 1.5 μM respectively (Fig. 7) for water column depths between the sea surface and nitracline depth. Huertas *et al*. (2005, 2006), Ruíz *et al*. (2006) and Prieto *et al.* (2006) observed similar low nitrate levels in summer in the Gulf of Cádiz. The autumn cruise represented a transitional situation between the prevailing oligotrophic conditions of the summer and spring cruises and the mesotrophic winter conditions. Nutrient levels were relatively high in association with relatively stronger continental inputs during this cruise.

Phytoplankton biomass, estimated according to the chl-*a* levels, also showed large seasonal and spatial variability. For the three zones, average chl-*a* levels were significantly higher during spring than for the other three cruises ($p<0.05$; Fig. 7). The lowest values were observed during the winter cruise for zone 1 and winter and summer cruises for zone 1 and 2 ($p<0.05$; Fig. 7). However, for zone 3 we did not observe any significant differences among the chl-*a* averages for summer, winter or autumn ($p<0.05$; Fig. 7). Previous studies, analysing sea surface distributions of chl-*a* in the Gulf of Cádiz, also described a similar seasonal pattern with maximum values close to the Guadalquivir River in spring and minimum values offshore in the winter months (Navarro and Ruiz 2006, Navarro *et al.* 2006, Prieto *et al.* 2009). In addition, with our data we have been able to resolve the vertical distribution of chl-*a* on a seasonal scale. The analysis of these distributions (Figs 5 and 6) reveal that maximum concentrations of chl-*a* were always subsurface and associated with nitracline depth for all cruises except winter when relatively high levels were reached at the sea surface at the 'oceanic' stations (zone 1).

Our results clearly show a large fluctuation in cyanobacteria cell abundance at the sea surface during the study year. *Prochlorococcus* cell abundance varied fourfold between the autumn and spring averages, while *Synechococcus* ranged between an average of 0.4±2.7 10^3 cells mL^{-1} (46±310 pg-C mL^{-1}) for summer and an average of 14.2±5.8 10^3 cells mL^{-1} (1595±648 pg-C mL^{-1}) for winter. The vertical locations of maximum cell abundance of the two cyanobacteria also followed different patterns. For *Prochlorococcus*, maximum cell abundance was always located at the sea surface except for the subsurface maximum at the SCM at the northern oceanic stations during summer. In contrast, *Synechococcus* maximum cell abundance shifted from maximum cell abundance at the sea surface during autumn and winter to maximum levels at SCM depth during summer and spring (Table 3). Thus, the magnitude and location of *Prochlorococcus* and *Synechococcus* maximum cell abundances were spatially separated during the summer and spring cruises as previously described for other oligotrophic systems (Olson *et al*. 1990; Partensky *et al*. 1996; Goericke *et al*. 2000) and also in the Gulf of Cádiz during a summer stratification scenario (Echevarría *et al*. 2009). However, our results also demonstrate that this 'complementary' pattern between *Prochlorococcus* and *Synechococcus* maximum cell abundances disappeared in the autumn and winter cruises when the system shifted to mainly mesotrophic conditions. Under these conditions maximum cell abundances for the two populations were observed in the oceanic sea surface waters (Fig. 4; Table 3). Thus, our study reveals that there is seasonal variability in the magnitude and location of maximum cell abundances for cyanobacteria populations in the Gulf of Cadiz that seems to be associated with the thermohaline and prevailing chemical conditions.

Thermohaline and chemical control in the chl-*a* and cyanobacteria distribution

Our study shows, for the first time, that there is a seasonal trend in the chl-*a* distribution not only at the sea surface but also in the vertical distribution associated with the thermohaline and chemical conditions. To summarize the control of the thermohaline and chemical conditions over the chl-*a* distribution, we made a diagram of average nitrate concentration vs water column stability for the three zones during the four cruises (Fig. 8). During winter the study ecosystem showed very low water column stability and the highest nitrate concentration due to water column

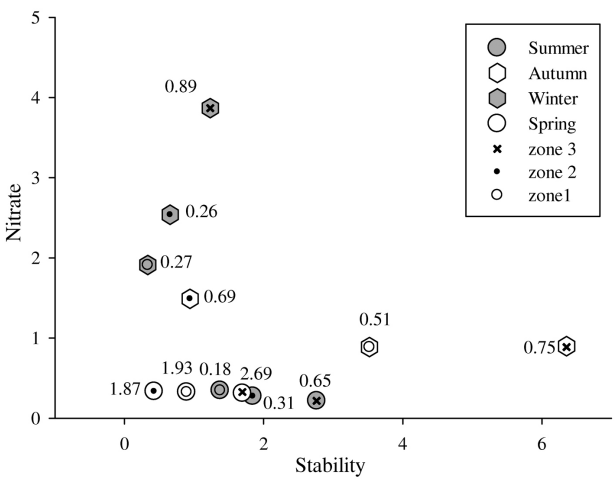

FIG. 8. – Relationship between the average integrated nitrate (μM) and average integrated water column stability from the sea surface to nitracline depth for the three identified zones (zone 2, zone 3 and zone 1; Fig. 1) in the Gulf of Cádiz during the summer, autumn, winter and spring cruises. Labeled numbers at each point correspond to the average integrated chl-*a* (μg dm^{-3}) from the sea surface to nitracline depth.

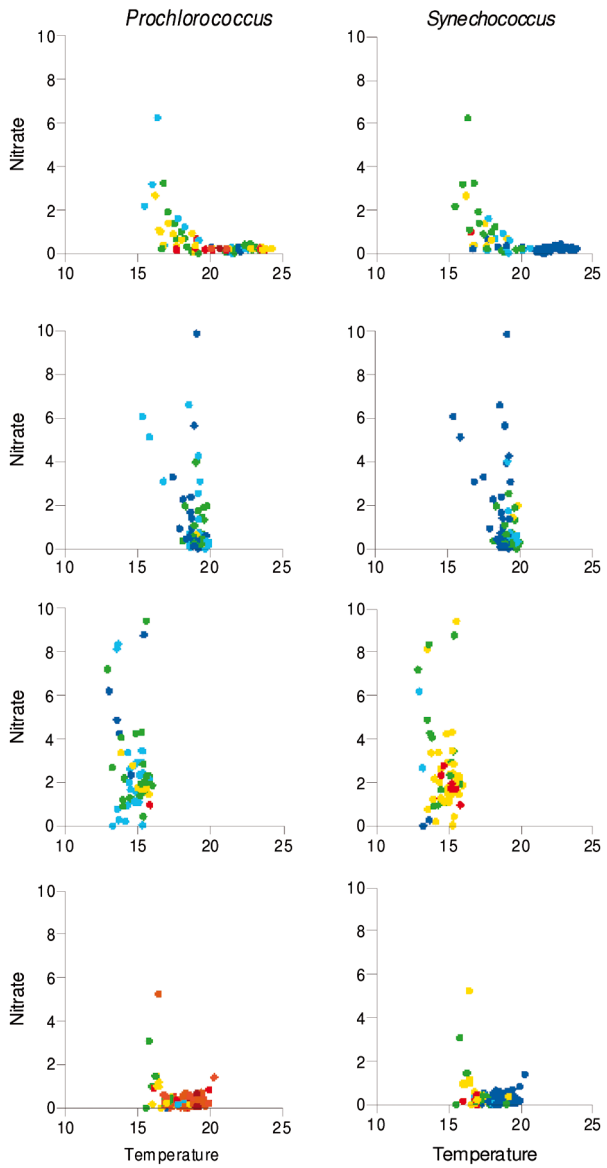

FIG. 9. – Relationship between the nitrate (μM) and temperature (°C) at the sea surface and SCM depth for the summer (a, b), autumn (c, d), winter (e, f) and spring (g, h) cruises. Dot colour scale corresponds to the cell abundance of *Prochlorococcus* and *Synechococcus* (cell mL^{-1}).

mixing in the oceanic zone and continental runoff in the inshore zones. Conversely, during summer, the strong temperature gradient due to solar heating favoured the establishment of a strong thermocline, and thus high water column stability. These warm waters are characterized by almost depleted nutrient concentrations. During the spring cruise, the water column showed relatively low stability, similar to the previous winter conditions, though with low nitrate levels, suggesting intense phytoplankton nutrient consumption. Finally, during autumn the water column stability was much higher in zone 3, due to the strong influence of the Guadalquivir River, than in the other two zones.

Therefore, based on our distributions of thermohaline and nutrient fields, and this summarizing analysis, the study system changed from a well-mixed eutrophic-mesotrophic system during winter to an oligotrophic scenario during the summer cruise. Following this thermohaline and chemical conditions, the phytoplankton biomass developed from minimum values during winter, due to light limitation in a well mixed water column, to maximum values in spring resulting from strong nutrient consumption. Subsequently, chl-*a* concentrations decreased when sea surface waters were almost nutrient-depleted. Navarro *et al.* (2006) also suggested that the SCM (or deep fluorescence maximum; DFM) observed in summer in the Gulf of Cadiz is related to the mechanism of winter mixing, when water masses are formed in this region. They established a conceptual model for this system (see Fig. 16 of Navarro *et al.* 2006) that developed from a well-mixed, high nutrient and low chlorophyll water column during winter to a well stratified, low nutrient, relatively low subsurface chlorophyll maximum in summer. The seasonal variability determined from our results strongly supports this conceptual model, indicating that the system evolves from eutrophic-mesotrophic conditions in winter to typical oligotrophic conditions in summer.

Prochlorococcus and *Synechococcus* cell abundances seem to be controlled, to some extent, by the thermohaline and chemical fields, as clearly illustrated in Figure 9. The highest cell abundances of *Prochlorococcus* were observed during the summer and spring cruises in association with warm temperatures (>15°C) and almost depleted nitrate levels; i.e, under typical oligotrophic conditions. In contrast, relatively low levels (>10 10^3 cell L^{-1}) were recorded during autumn even though the water temperature was >18°C and nitrate levels ranged from 0 to 6 μM. During winter, when the water temperature was low (<15°C) and nitrate levels varied between 0 and 10 μM, *Prochlorococcus* cell abundances were also low. The *Synechococcus* population seems to show the highest cell abundances in association with cold temperatures and relatively high nitrate levels. In fact, the highest cell abundances were recorded during winter and the lowest values in the warm and nutrient-poor waters of the summer and spring cruises. Previous studies (Chisholm 1992, Jacquet *et al.* 2002) also indicated that *Prochlorococcus* are more abundant under more typical oligotrophic conditions, as observed in our summer and spring cruises, while *Synechococcus* prevail under more mesotrophic conditions, as occurred during our winter cruise. Overall, there is a seasonal fluctuation not only in the cell abundance of cyanobacteria but also in the contributions of *Prochlorococcus* and *Synechococcus* in association with the different oceanographic conditions. Further studies with a higher temporal and spatial (vertical) resolution are necessary to clearly assess these controlling factors.

CONCLUSIONS

In summary, the results presented in this study show seasonal variability of chl-*a* and cyanobacteria populations (*Prochlorococcus* and *Synechococcus*) in the NE region of the Gulf of Cádiz. The spatial and seasonal distributions of these variables are regulated by the thermohaline and chemical properties observed during each of the four seasonal cruises. Based on our observations, it seems that the system varies from mesotrophic conditions with low phytoplankton biomass due to winter mixing conditions, to maximum chl-*a* levels associated with strong nutrient consumption in spring and then shifts to an eutrophic scenario with almost depleted nutrients in summer. *Prochlorococcus* and *Synechococcus* followed two different distribution patterns. *Prochlorococcus* showed the highest abundance during the summer and spring cruises in association with warm temperatures and depleted nitrate levels. In contrast, low levels were recorded during autumn and winter, when the water temperature was low and nitrate levels were high. The *Synechococcus* population showed maximum cell abundances in association with cold temperatures and relatively high nitrate levels. In fact, the highest cell abundances were recorded at the sea surface during winter.

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